بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 6

DNA Replication pt.2

Written by : DST
Hashem Alhalalmeh

Reviewed by : Hashem Alhalalmeh

وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: الذي له ملك السموات والأرض ومن فيهنّ، لا ملك فوقه، ولا شيء إلا دونه، له الملك المطلق، والتصرف في الكون كله، وهو المالك لكل شيء، والمليك دالٌّ على عظيم ملكه تعالى.

الورود: ورد اسم الملك (٥) مرات، أما اسم المليك فورد مرة واحدة، واسم المالك مرتان.

الشاهد: ﴿ٱلْمَلِكُ ٱلْقُدُّوسُ﴾ [الحشر:٢٣]، ﴿فِي مَقْعَدِ صِدْقٍ عِندَ مَلِيكٍ مُّقْتَدِرٍ﴾ [القمر:٥٥]، ﴿قُلِ ٱللَّهُمَّ مَٰلِكَ ٱلْمُلْكِ﴾ [آل عمران:٢٦].

اضغط هنا لشرحٍ أكثر تفصيلًا

Last Lecture – Quick Review

- Semi-conservative: Each daughter DNA gets one old (template) and one new strand. Conservative & dispersive are other models but later were proved wrong.

- Direction: Bidirectional from origin; synthesis 5'→3', template read 3'→5'.

- Strands:

Leading strand: Continuously synthesized.
Lagging strand: Discontinuous; Okazaki fragments. Fragments need primers, later joined by ligase using ATP.

- Primers & Enzymes:

Primase: Synthesizes short RNA primer (ribonucleotides, uracil instead of thymine).

- DNA Polymerases:

Bacteria: Pol III synthesizes until RNA primer; Pol I removes RNA (5'→3' exonuclease) and fills gaps.
Humans: Pol δ fills gaps; RNAse H removes primers; ligase joins fragments.

Last Lecture – Quick Review

Accessory Proteins:

- Sliding clamp (PCNA in humans): Stabilizes polymerases, loads them onto primers; diagnostic marker for proliferating cells, especially used in cancer cells detection.

- DNA helicase: Unwinds DNA, creating single-stranded templates.

- SSBPs / Replication Protein A: Bind ssDNA, protect from degradation, prevent hairpins and renaturation.

- Topoisomerases: Relieve supercoiling.

Type I → cuts one strand
Type II → cuts both strands; required for chromosome condensation; also, in sister chromatids separation; target for cancer therapy.

How accurate is DNA replication?

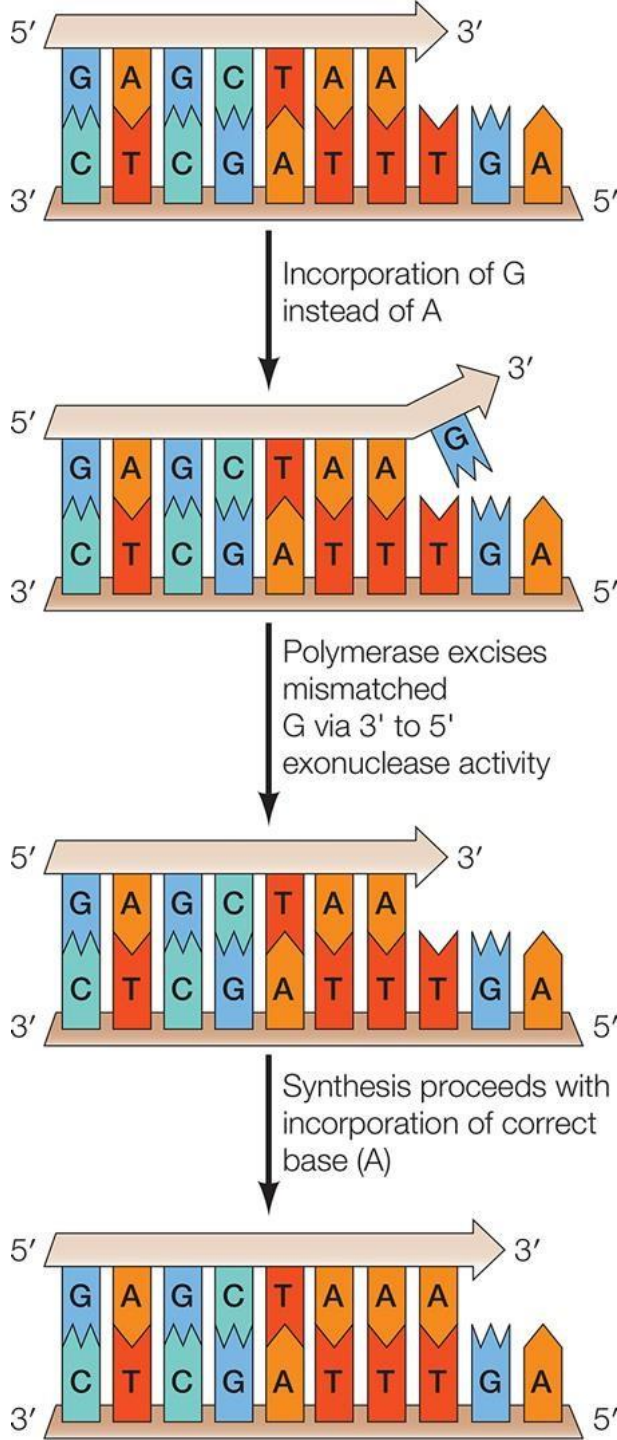
- **DNA replication MUST be accurate, mistakes are not tolerated, as it is the basis of life.**

- The frequency of errors during replication is only one incorrect base per 10^9 nucleotides incorporated.

- How is accuracy high?
  - The DNA polymerase can catalyze the formation of the right phosphodiester bonds between the complementary bases with the proper hydrogen bonding (accuracy=10^{-5}).
  - Proofreading mechanism (a 3'→5' exonuclease activity) increasing the accuracy to 10^{-8}.
  - Repair mechanisms (to be discussed later)

Note:

- **Accuracy = 10^{-5}:** DNA polymerase makes 1 mistake per 100,000 nucleotides added (before proofreading).
- **Proofreading (3'→5' exonuclease activity):** Corrects most mistakes as they occur, increasing fidelity from $10^{-5} \rightarrow 10^{-8}$ (1 mistake per 100,000,000 nucleotides).



Replication fidelity:
Error rate: ~1 mistake per 1,000,000,000 nucleotides.
Human genome: 3,000,000,000 nucleotides per haploid genome → 6,000,000,000 in diploid cells (all 23 chromosome pairs).
Therefore, ~6 mistakes per replication per cell. Extremely accurate.

Where does DNA replication fidelity come from?

1.Properties of the enzyme: DNA polymerases are highly specific enzymes. When the polymerase encounters an A on the template strand, it incorporates the complementary T. Occasionally, it might try to insert a wrong nucleotide, like C, but the active site of the enzyme won't allow it to fit properly. Because of the shape and hydrogen bonding requirements—A forms 2 H-bonds, C forms 3—the enzyme detects the mismatch and removes the incorrect nucleotide, replacing it with the correct one. Essentially, the enzyme "senses" which bases fit together properly.

2.Proofreading mechanism: DNA polymerases have an in-built proofreading function. Imagine the enzyme writing quickly across 50 pages—when it makes a mistake, it uses an "eraser" to correct it. This is due to the exonuclease activity in addition to polymerase activity. In bacteria, DNA polymerase I has 5'→3' exonuclease activity, but in humans, the exonuclease activity works in the 3'→5' direction. This allows the enzyme to remove wrongly incorporated nucleotides and replace them with the correct ones during replication.

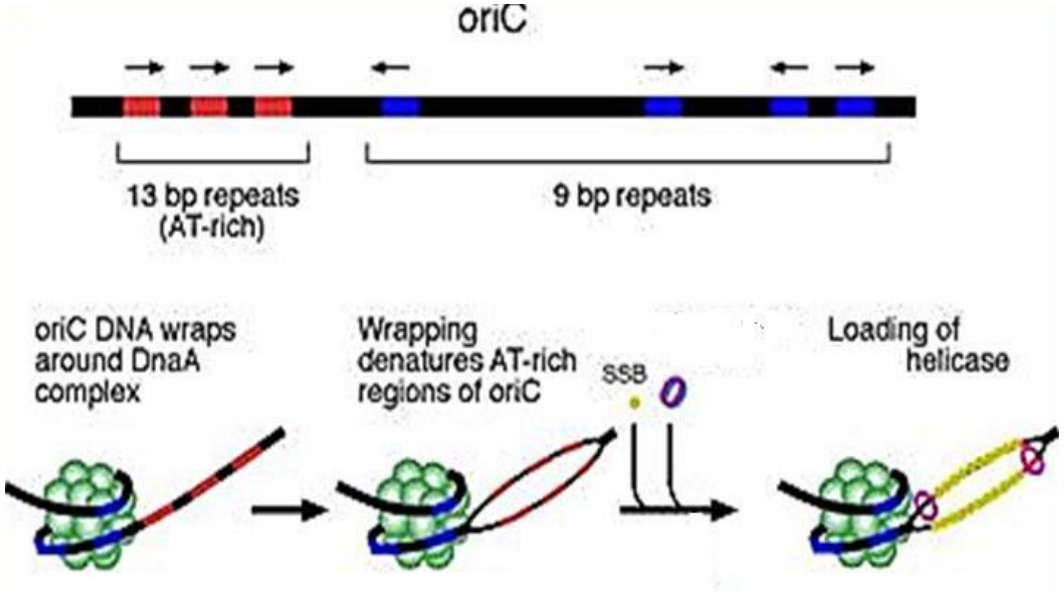
3.Repair mechanisms: After replication, additional repair systems correct any remaining mistakes, further ensuring fidelity.
Cool fact: Every cell accumulates 10,000-100,000 errors daily, but repair mechanisms are constantly working to fix them, 24/7.

Origin of replication (OriC) in bacteria

- Bacterial replication starts at an origin of replication (OriC).
- oriC regions contain repetitive 9-bp and AT-rich 13-bp sequences (These are **consensus sequences** which are preserved DNA sequences, found in different DNA molecules of different species. A consensus sequence of genes means the same sequence is found in different genes, These sequences are important, and they have different functions).
- 9-mer: binding sites for DnaA protein. Not tandem, but are in the same locus
- 13-mers: Tandem AT-rich region - it facilitates separation of the double-stranded DNA.

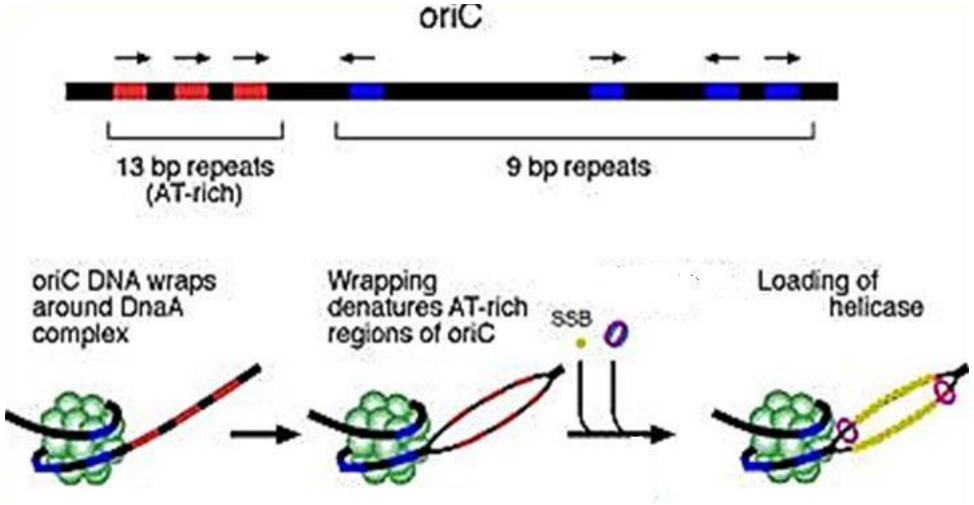
Mer =unit,
Just like octamer, dimer etc.

- DnaA protein binds to 9- mers, applies stress on the AT-rich region, and OriC opens up, because of the weak H-bonding in the AT region, instead of GC rich regions.
- As soon as a ssDNA region appears, The helicase, SSB proteins and primase etc jump on, followed by the replication machinery.



Origin of replication (OriC) in bacteria, Further explanation

- To explain the repetition pattern:
- Look at this diagram, it shows that inside of the OriC region there 2 consensus sequences that are repeated & function as



- follows:

9mer sequence aka 9 base pairs:

- This consensus sequence is repeated several times no n-tandemly in different orientations within the short OriC region.
- It functions as binding sites for the protein DnaA.
- After binding to the 9mers, DnaA wraps the DNA around it, then it exerts pressure on 13mers(which ha ve weak interactions between dsDNA)and so, this pre ssure will cause the AT-rich 13mer region to open up for replication to start.

Analogy: Think of a balloon: if you press on one end of the ballon, the other end will inflate.

- **13mer sequence aka 13 base pairs:**
- This consensus sequence is repeated **tandemly** in one direction within OriC.
- **Tandemly** repeated means repeated one after the
- other with no interruptions.
- These sequences are AT rich, with 2 hydrogen bonds between A's & T's, so the interaction between the
- two strands isn't very strong.
- This weak interaction functions in facilitating the separation of dsDNA.
- After the opening of OriC, other molecules will jump
- on the open DNA to create the replication bubble

Origins of Replication – Human Genome

- **Do humans have origins of replication?**

Answer: Yes, each chromosome contains multiple origins of replication.

- **Why do human chromosomes have multiple origins?**

Answer: The human genome is large, so multiple origins ensure replication completes efficiently.

- **Do human origins** have 9-mers or AT-rich 13-mers like bacteria.?

Answer: It's not obvious, until this moment we don't quite know the origins of replication in human DNA.

- **Do we know the exact locations of human origins?**

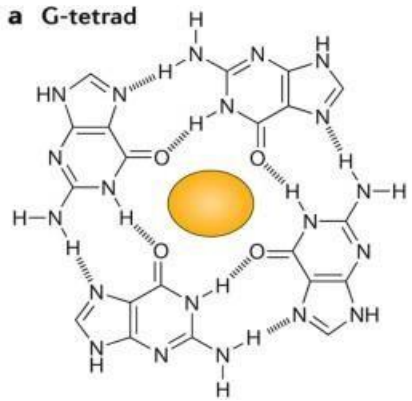
Answer: No, the precise locations are unknown, but some features have been identified that may help locate them.

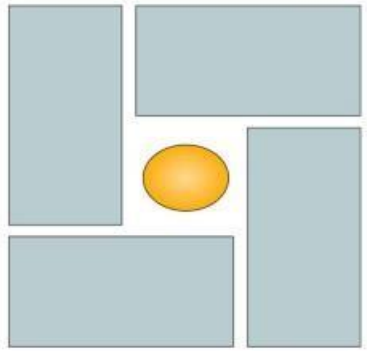
Origins of replication in the human genome

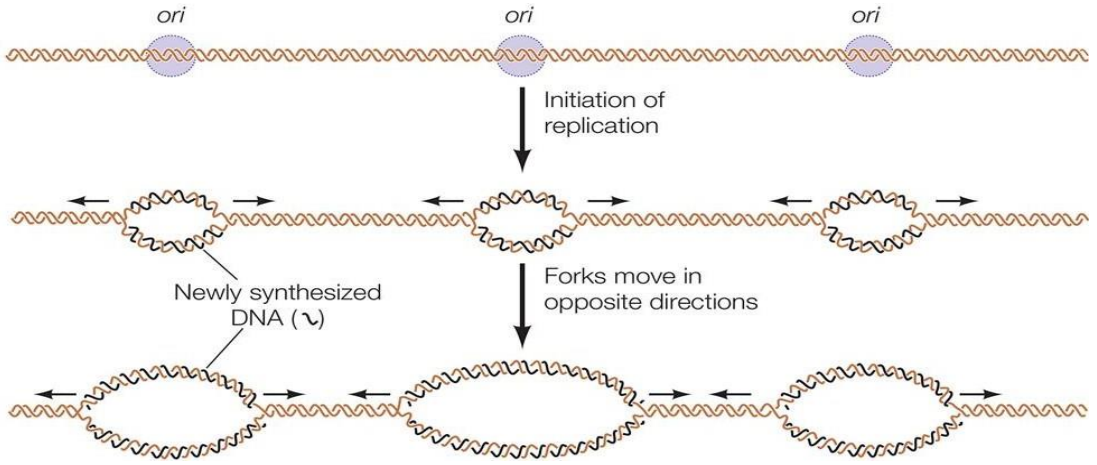
- The human genome has about 30,000 origins of replication with the following characteristics/features:
  - G-rich sequences that can form G-quadruplex secondary structures. (4 G nucleotides form hydrogen bonds with each other.)
  - **Modified** histones that promote chromatin **decondensation** and activation of gene expression. The modification compromises the interaction between histones and DNA and weakens it, thus DNA opens up more easily.
  - Close proximity to actively transcribed gene, to ensure that the important genes are replicated earlier than inactive ones.
  - Cell-specific, origins of replication in the human DNA are different according to the cell type, because different cells have different active genes.

All of them work at a reasonably similar time. And since DNA replication is bidirectional, eventually they will meet each other, and fusion of newly-synthesized DNA will take place.

This is a G-quadruplex



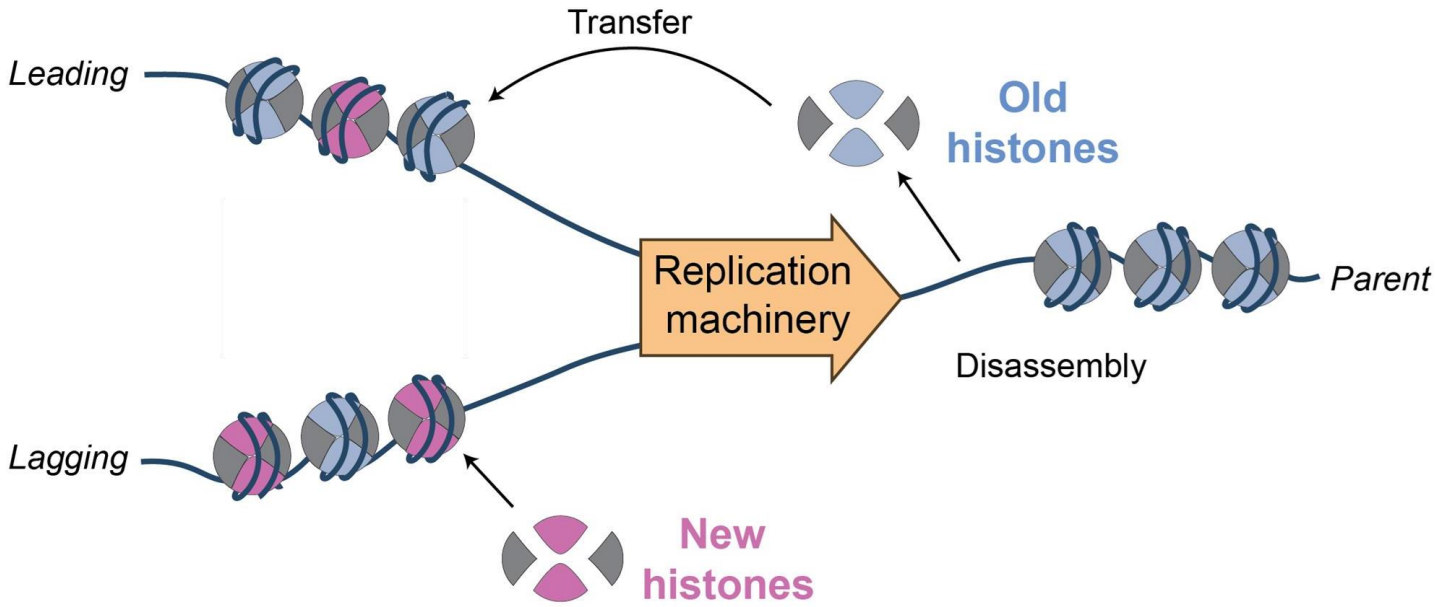




The formation of nucleosomes

Problem #1

- In the human genome, there are 4 genetic clusters containing a total of 65 histone-coding genes.
- Nucleosomes are disassembled and reassembled during DNA replication by histone chaperones, which use recycled and newly synthesized histones.



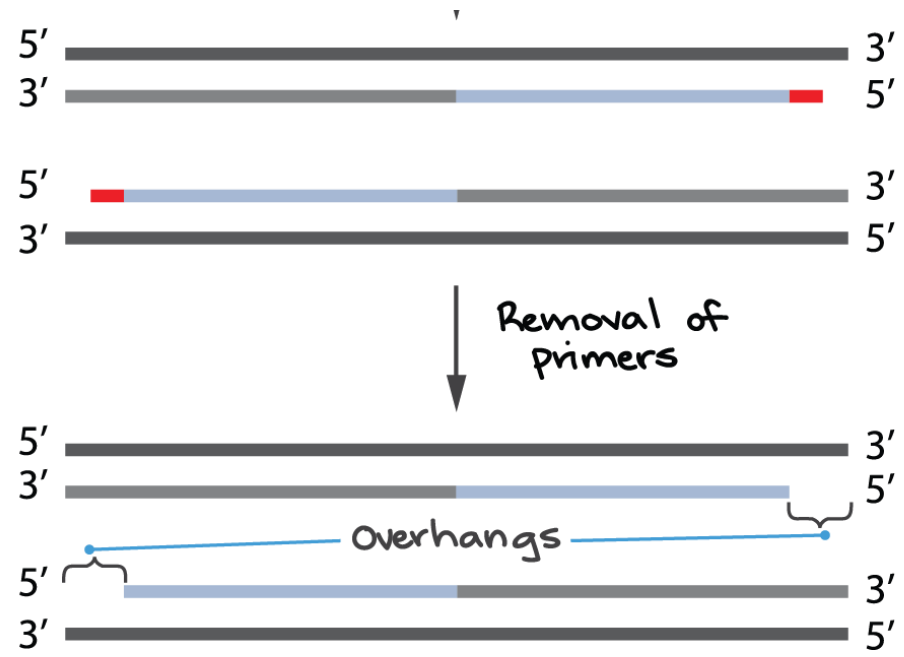
The formation of nucleosomes

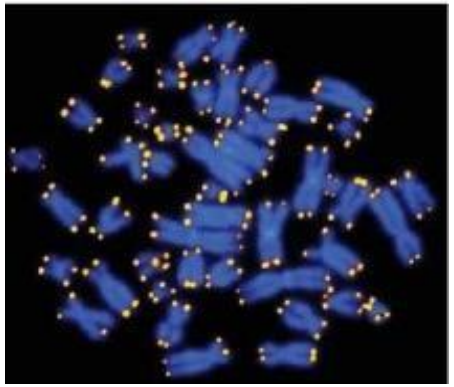
- This process does not exist in bacteria because only **eukaryotic DNA** is organized as **chromatin** (DNA complexed with histones).
- As DNA doubles during replication, histones must also double to maintain proper chromatin structure. To achieve this, histones are synthesized via **transcription and translation**, processes that **cannot occur simultaneously** with DNA replication.
- To solve this problem, there are **4 genetic clusters** containing **65 histone-encoding genes**. This allows DNA to be replicated while histone proteins are being produced from different DNA regions (the clusters) that are not currently being replicated.
- There is **continuous production of histones**, as the 65 histone-encoding genes are actively transcribed.
- During DNA replication, **histones are removed and then re-added** (assembly and disassembly of nucleosomes). This process is facilitated by **histone chaperones**.
- Histone chaperones can:
- Recycle **old histones** removed during nucleosome disassembly and assemble them into new nucleosomes.
- Take **new histones** and assemble them onto the newly replicated DNA.

A problem in the lagging strand

Problem #2

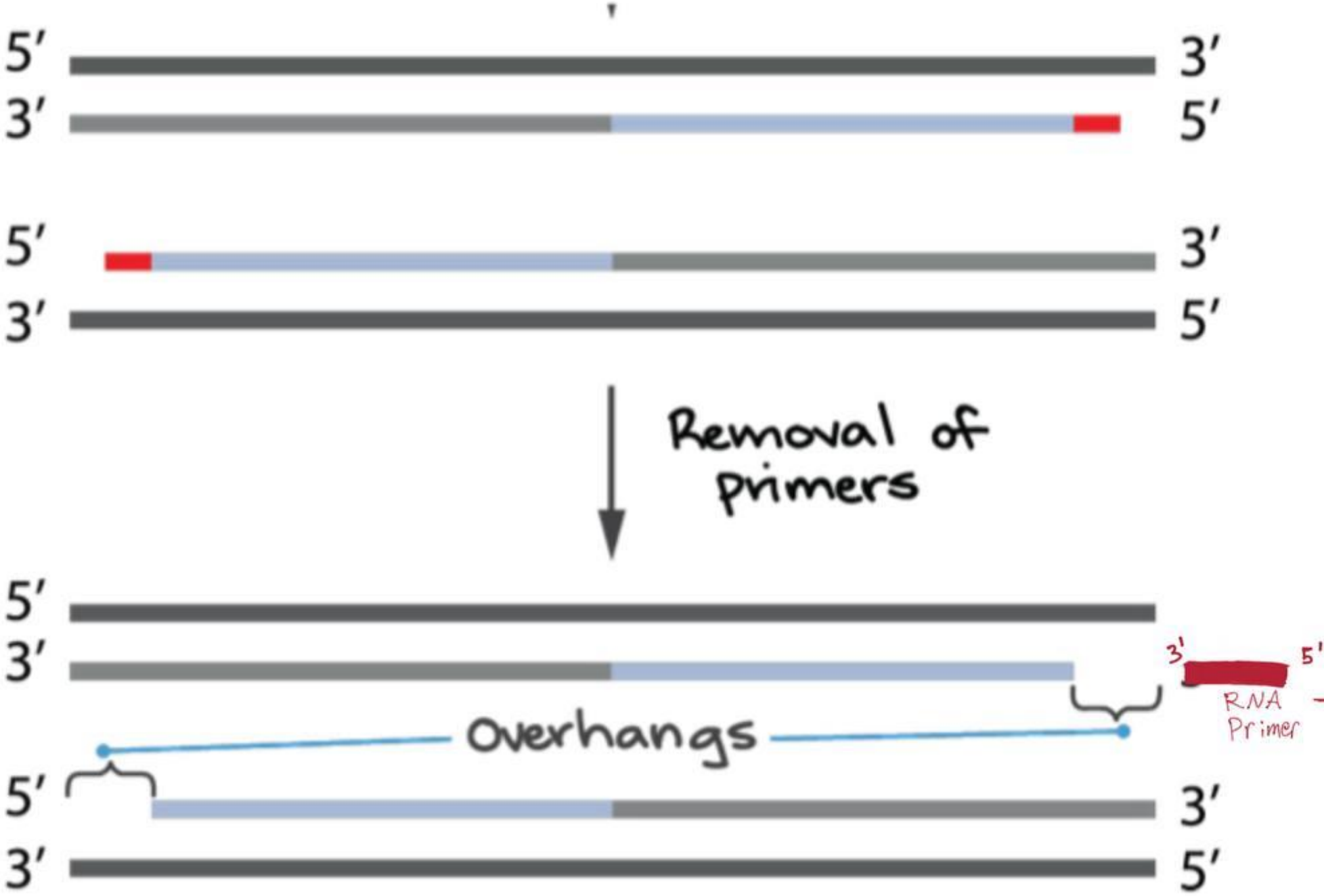
- Since DNA is synthesized in an antiparallel manner, the lagging strand synthesis faces a challenge: after primer RNA is added, and okazaki fragments are synthesized and ligased, then:
- As the growing fork approaches the end of a linear chromosome, the lagging strand is not completely replicated. Why?
- When the final RNA primer is removed, there is no place onto which the DNA polymerase can fill the resulting gap leading to the shortening of the lagging strand.
- This problem is only in the lagging strands and it happens at the 2 ends of the chromosome





FISH technique.

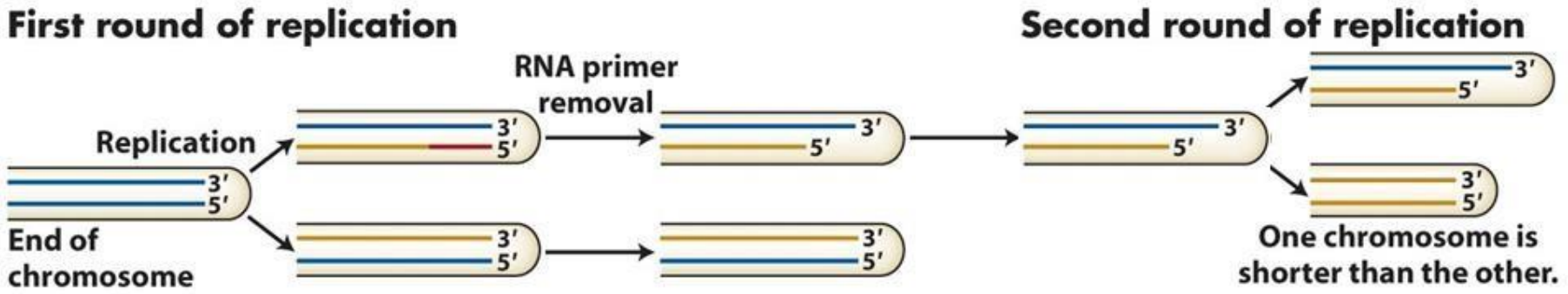
"No room" for DNA polymerase to fill the gap happens because there's No upstream 3′ hydroxyl group available to replace the final RNA primer with DNA, as DNA polymerase can only add in 5' to 3' direction, but it needs a free OH group from a free 3' end to do such.



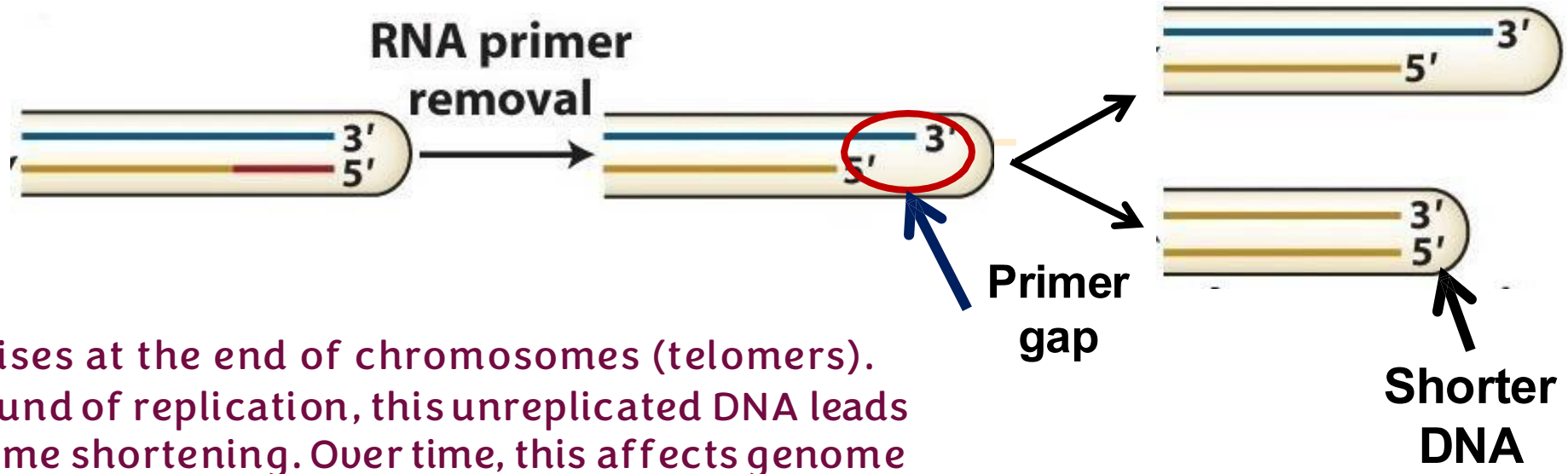
We would need a primer floating here in order for DNA Polymerase to add new nucleotides to fill this gap.

Really hope this can help to imagine the problem

The previous slide is just me trying to make it easier to **visualize** the problem we are facing.
It's not actually the solution of the problem because primer RNA can never float and be stable like this.
The real solution is in the following slides.
So please don't get confused, and good luck.



Notice the 3' overhang, the end of DNA is a single-stranded 3' end, that loops around sheltrin proteins.



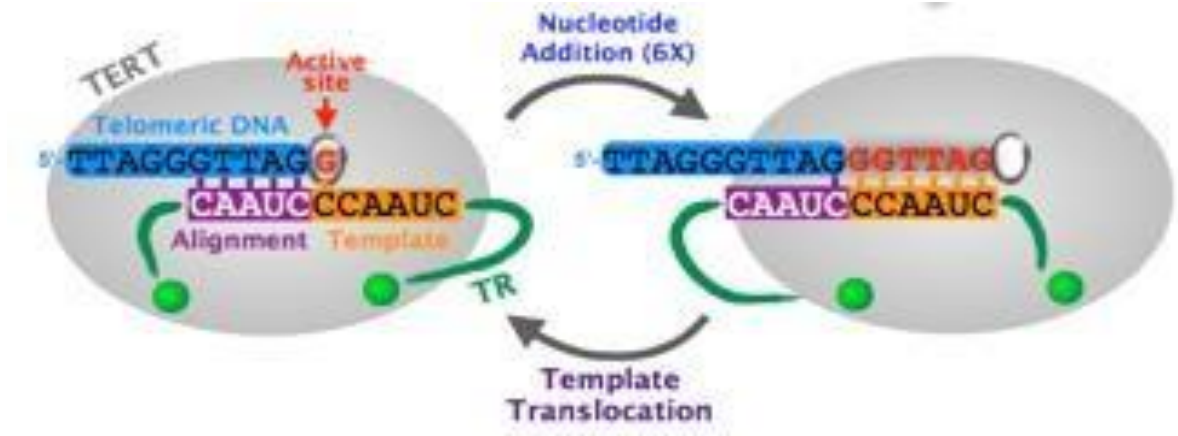
This issue arises at the end of chromosomes (telomers). With each round of replication, this unreplicated DNA leads to chromosome shortening. Over time, this affects genome stability, making chromosomes more prone to mutations, breaks, and damage. This process contributes to aging, as shorter chromosomes destabilize cells and lead to cell death. REMEMBER: telomers are important for DNA stabilization.

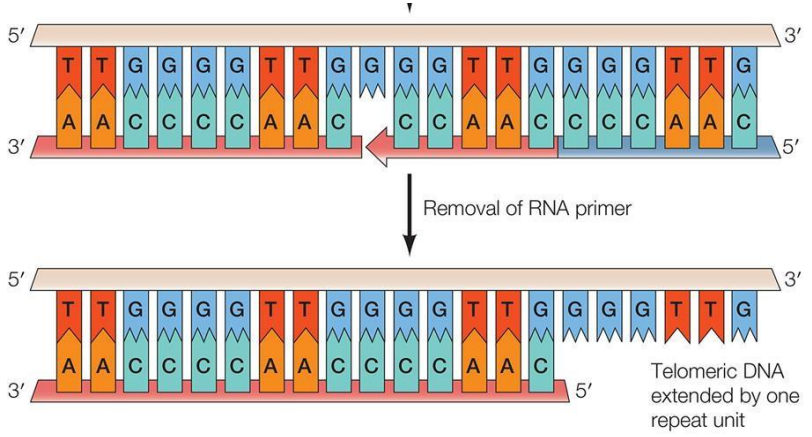
Chromosome shortening is linked to aging. As we age, chromosomes get shorter and shorter, destabilizing cells. This process raises a question: why are we not dead before birth, given the extensive DNA replication during development? (next slide)

Telomerase comes to the rescue

The solution

- An enzyme (reverse transcriptase) that elongates the lagging strand.
- Telomere DNA sequences consist of many GGGTTA repeats extending about 10,000 nucleotides.
- Telomerase (**a reverse transcriptase**) prevents the progressive shortening of the lagging strand. How?
- Telomerase elongates it in the 5'-to-3' direction using **an RNA template** (primer) that is a component of the enzyme itself. This makes it a ribonucleoprotein (a holoprotein, part of it composed of protein and the other part of RNA)
- When the last primer is removed, a 3'-overhang is left. (next slide for further explanation)



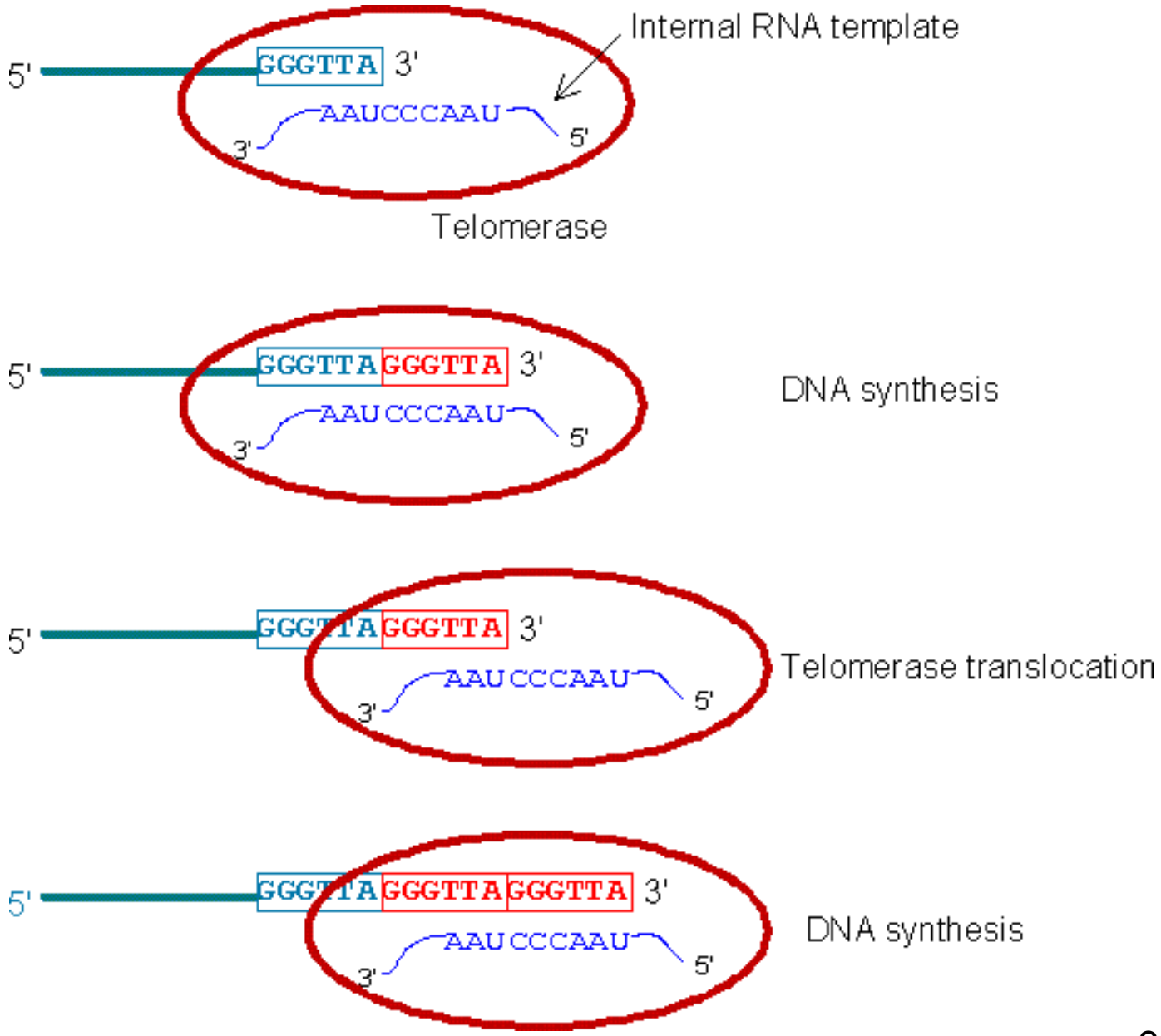


Telomerase enzyme uses its primer to elongate DNA. This is why it's a reverse transcriptase (uses an RNA template to synthesize DNA).

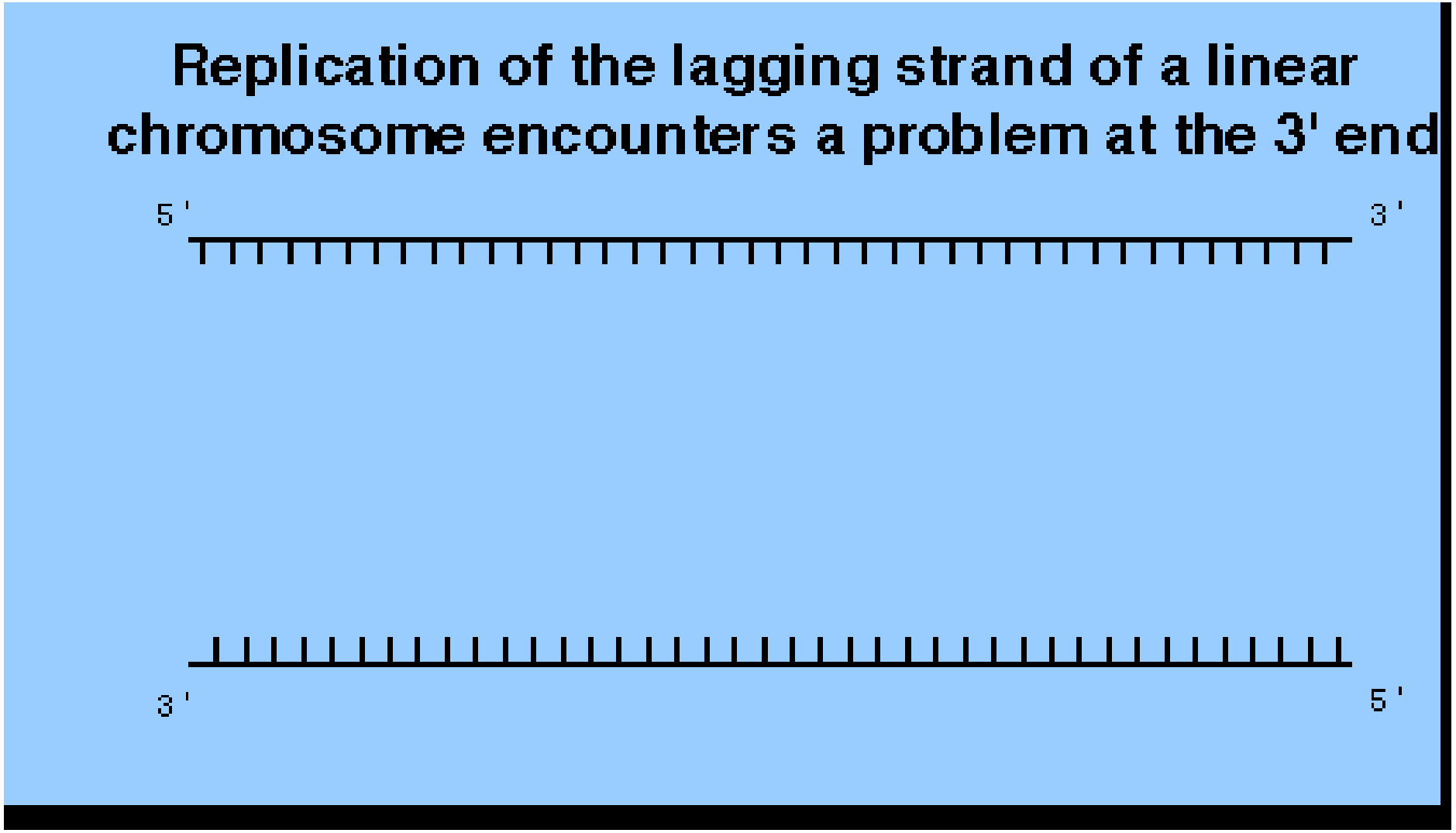
Mechanism of Action:

- **Telomerase** binds to 3' overhang and elongates the DNA at the telomere by repeating the sequence GGGTTA (which characterizes telomeres) multiple times.
- Once the DNA is elongated, **primase** has enough space to add a new primer, enabling **DNA polymerase** to synthesize another Okazaki fragment.
- This process keeps the DNA at telomeres long enough to stabilize the chromosome.
- **Telomeres** end with a **3′ overhang**, which is single-stranded and loops around proteins like **shelterin** to form a protective cap.
- This looping protects chromosome ends from being recognized as DNA breaks.

(discussed in the genome lecture)



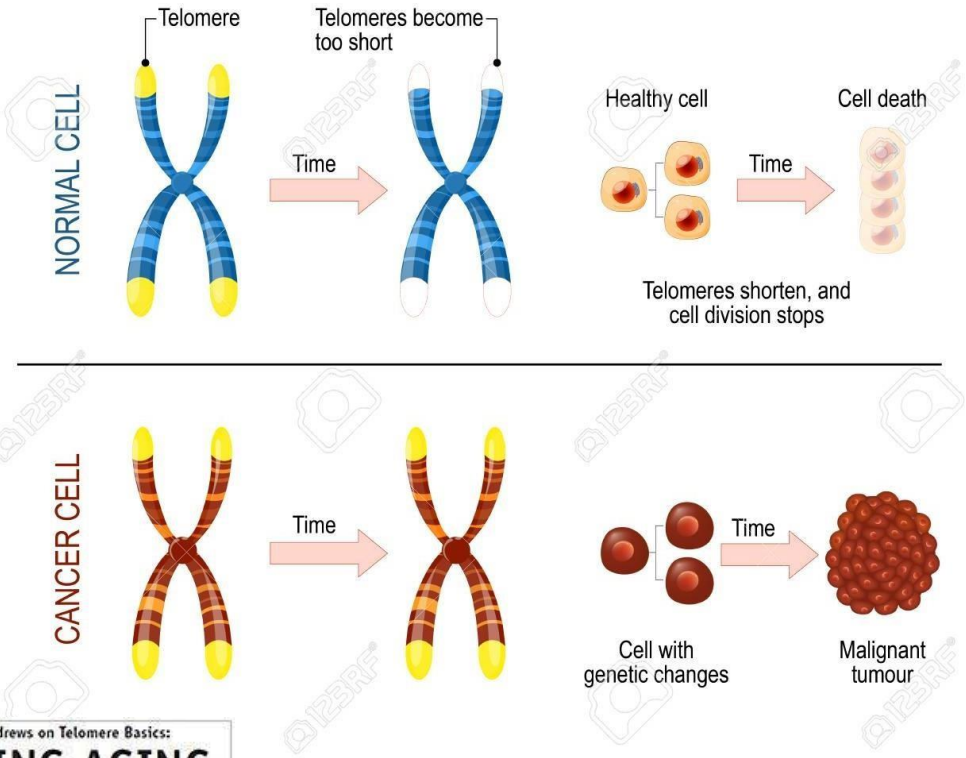
Link to animation :)

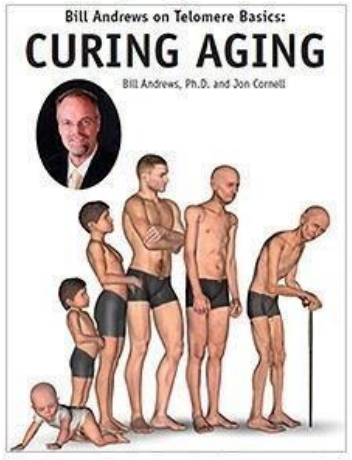


Note: Although this animation is good, there are wrong pieces of nformation within it. Find them.

Facts of life about telomerases

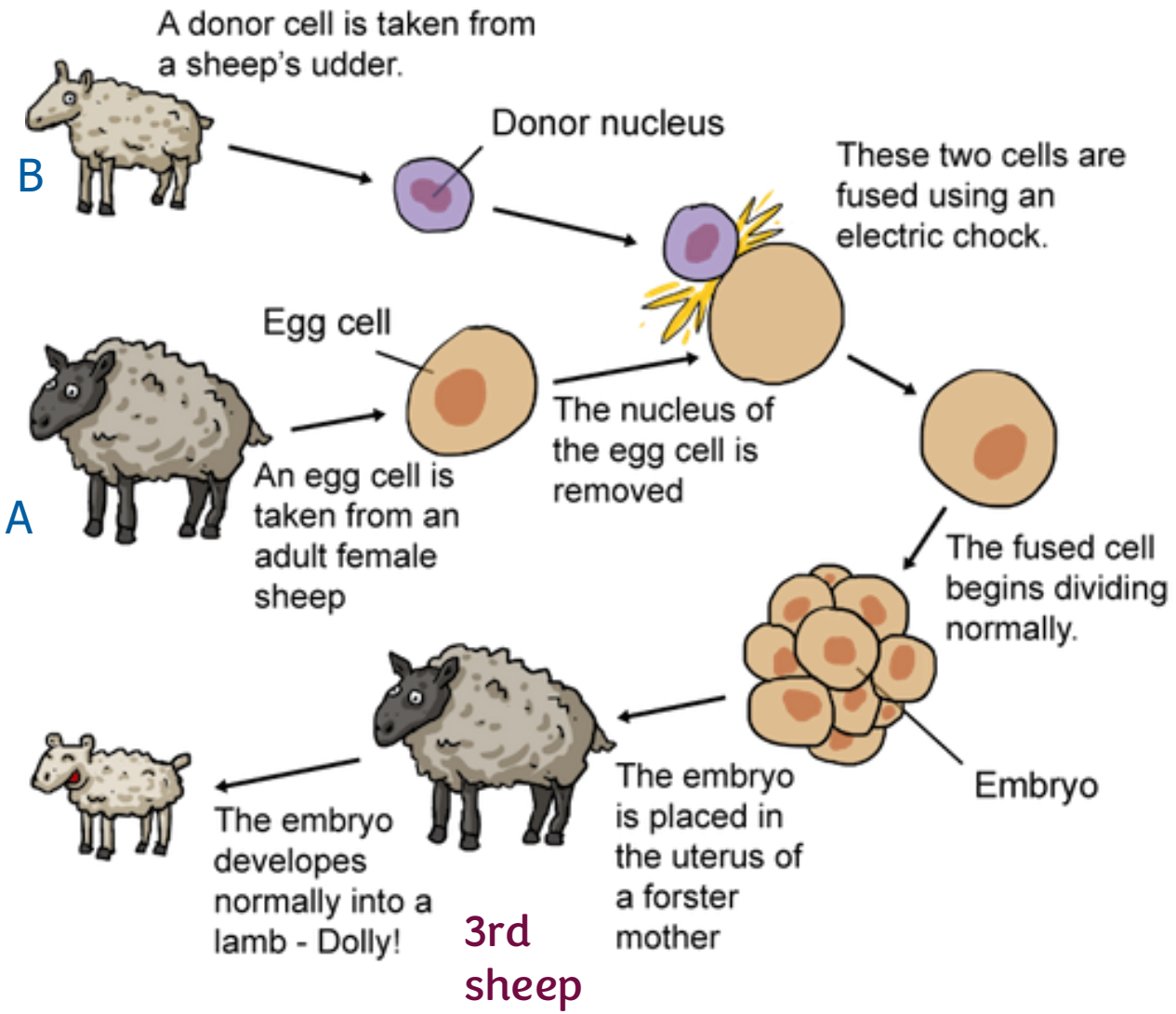
- Most somatic cells do not have high levels of telomerase and, hence, have a finite number of cell divisions.
- As we grow older, the **levels** and **activity** of telomerase are reduced.
- The gradual shortening of the chromosome ends leads to senescence and cell death.
- Germline (stem cells) and cancer cells express high levels and activity of telomerase. This is what makes them immortal. As chromosomes do not get shorter as they divide.





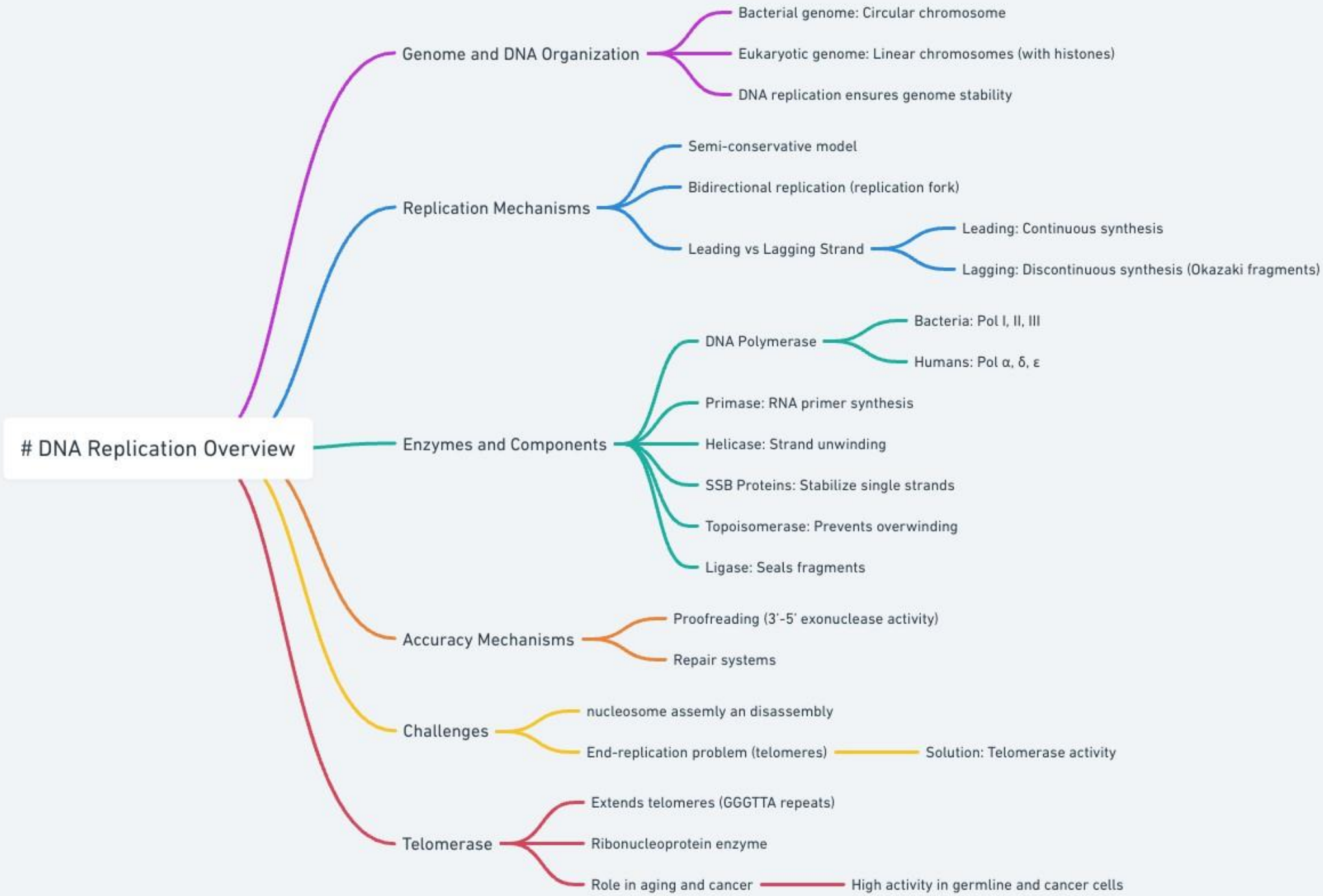
Dolly, the sheep

The first mammal ever to be cloned.



Dolly lived for 6.5 years instead of the normal **11-12 years**.

Scottish scientists took an egg from a donor "A" and stripped it from its nucleus.
Then a nucleus from a cell was taken from another donor "B". The nucleus taken from donor "B" was inserted into the egg cell from donor "A" and the egg was fertilized.
The resulting zygot was implanted within a third sheep, and dolly came out to be a clone of donor "A" that gave up its genetic information to dolly.
Interesting to note that, the DNA taken from donor "A" was 6 years old, so the telomeres were already shortened, this was thought to be a contributing factor to why dolly lived shorter (her DNA was already 6yrs old and she lived for 6 additional years)
But it turns out this wasn't the case :) dolly died because of an infection.


DNA Replication Overview
Genome and DNA Organization
Bacterial genome: Circular chromosome
Eukaryotic genome: Linear chromosomes (with histones)
DNA replication ensures genome stability
Replication Mechanisms
Semi-conservative model
Bidirectional replication (replication fork)
Leading vs Lagging Strand
Leading: Continuous synthesis
Lagging: Discontinuous synthesis (Okazaki fragments)
Enzymes and Components
DNA Polymerase
Bacteria: Pol I, II, III
Humans: Pol α, δ, ε
Primase: RNA primer synthesis
Helicase: Strand unwinding
SSB Proteins: Stabilize single strands
Topoisomerase: Prevents overwinding
Ligase: Seals fragments
Accuracy Mechanisms
Proofreading (3'-5' exonuclease activity)
Repair systems
Challenges
nucleosome assemly an disassembly
End-replication problem (telomeres)
Solution: Telomerase activity
Telomerase
Extends telomeres (GGGTTA repeats)
Ribonucleoprotein enzyme
Role in aging and cancer
High activity in germline and cancer cells

رسالة من الفريق العلمي:

معرفتنا إنو في يوم رح تنتهي حياتنا ما لازم تخوّفنا، بالعكس... هي تذكير إن الوقت محدود، وإنه أحسن نستغله بالخير، عشان نلقى ربنا بقلب مرتاح وبعمل يرضيه.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 13 & Slide 21	"no room" for DNA polymerase to fill the gap happens because there's No upstream 3′ hydroxyl group available to replace the final RNA primer with DNA, as DNA polymerase can only add in **3' to 5' direction.**	"No room" for DNA polymerase to fill the gap happens because there's No upstream 3′ hydroxyl group available to replace the final RNA primer with DNA, as DNA polymerase can only add in **5' to 3' direction, but it needs a free OH group from a free 3' end to do such.** **At slide 21 the 2 sheep (A&B) got flipped**
V1 → V2			